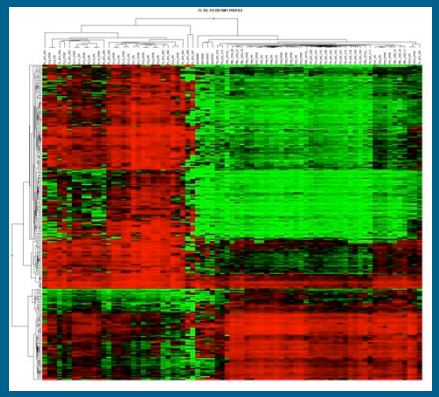
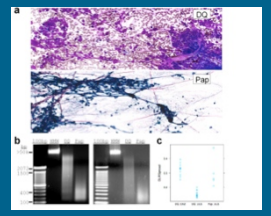
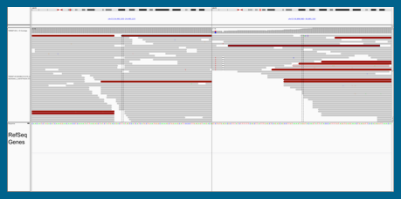
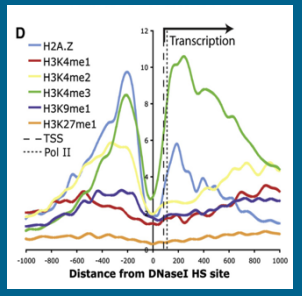
NEW ADVANCES IN CANCER DIAGNOSIS

Paul Meltzer
Genetics Branch
CCR/NCI









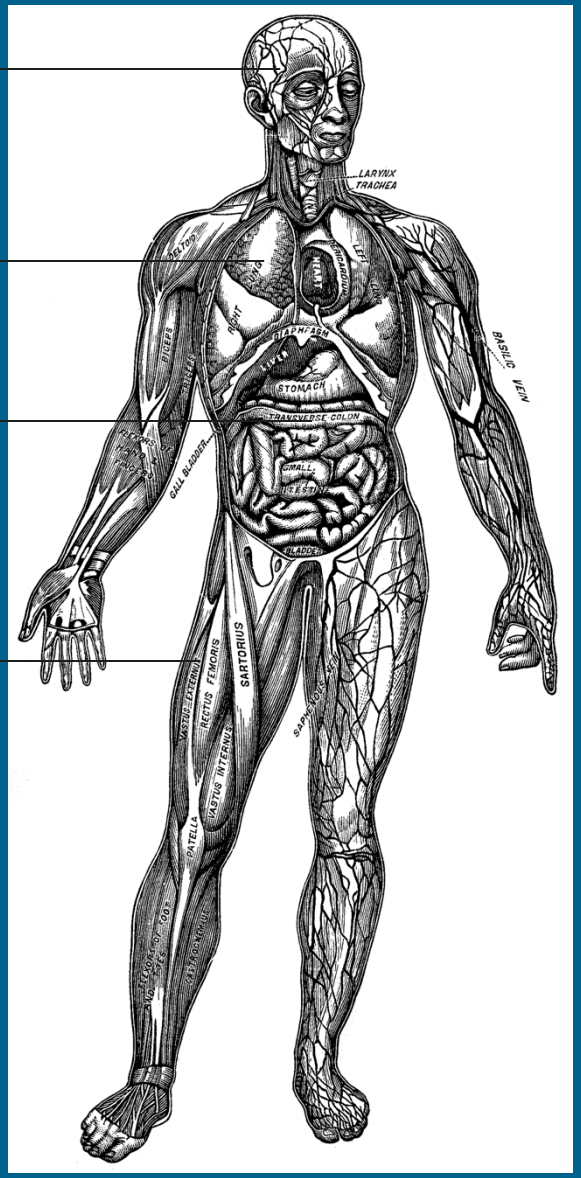
DIAGNOSIS: ANATOMY

BRAIN CANCER

LUNG CANCER

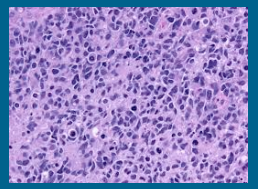
COLON CANCER

MUSCLE CANCER

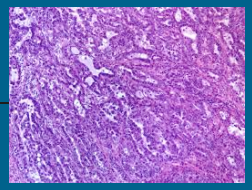


DIAGNOSIS: HISTOLOGY

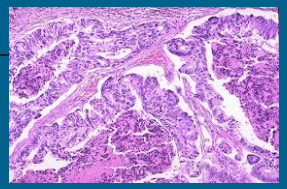
BRAIN CANCER



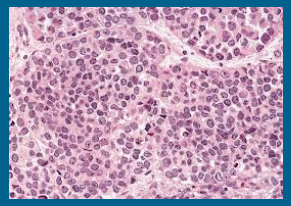
LUNG CANCER



COLON CANCER

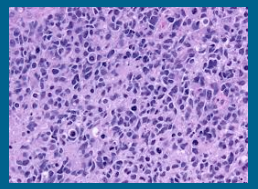


MUSCLE CANCER



DIAGNOSIS: HISTOLOGY

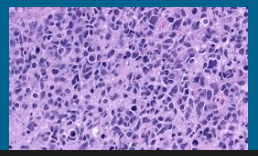
BRAIN CANCER



- DEFINITIVE DIAGNOSIS OF CANCER
- RECOGNITION OF SUBTYPES
- PLATFORM FOR CONTINUOUS DIAGNOSTIC REFINEMENT
- BASIS OF ALL CLINICAL DECISION MAKING
- BASIS OF ALL CLINICAL CANCER RESEARCH

DIAGNOSIS: HISTOLOGY

BRAIN CANCER



QUESTION:

HAS THE PROGRESSIVE SUBDIVISION OF CANCERS INTO PRECISELY DEFINED ENTITIES DISTRACTED US FROM THE POTENTIAL IMPORTANCE OF COMMONALITIES AMONG TUMORS WHICH CUT ACROSS DIFFERENT HISTOTYPES?

NEW ADVANCES IN CANCER DIAGNOSIS BASED ON THE EVER DEEPENING UNDERSTANDING OF CANCER BIOLOGY AND ADVANCEMENTS IN BIOTECHNOLOGY

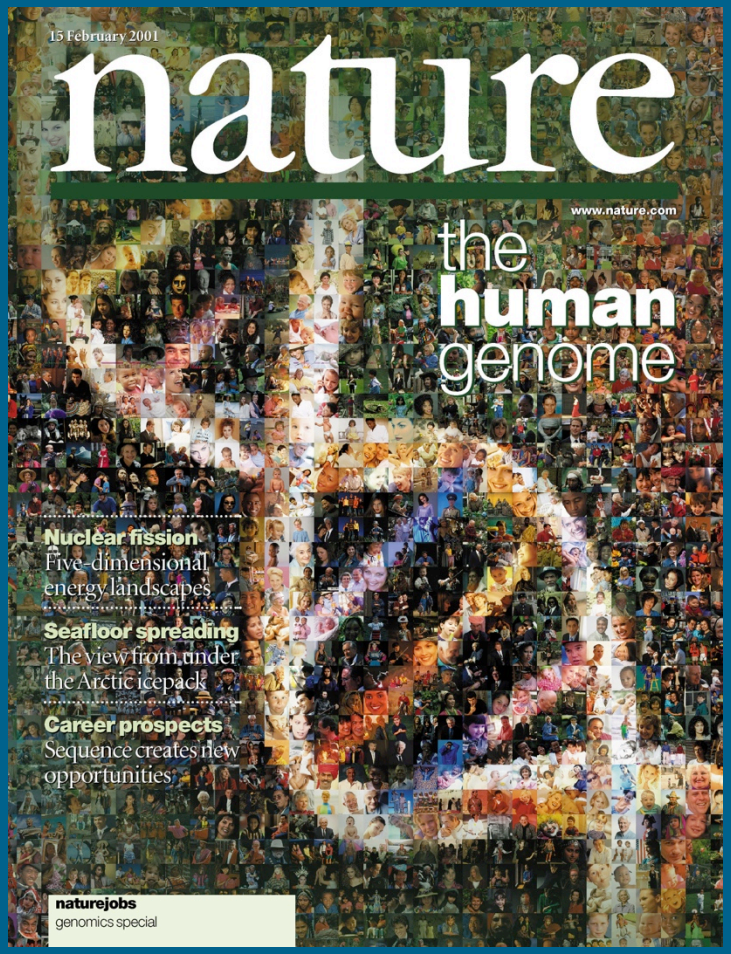
GOALS

- BIOLOGICALLY INFORMED

  -CANCER DIAGNOSIS
  -CLINICAL DECISION MAKING

- PRECISION CANCER THERAPY

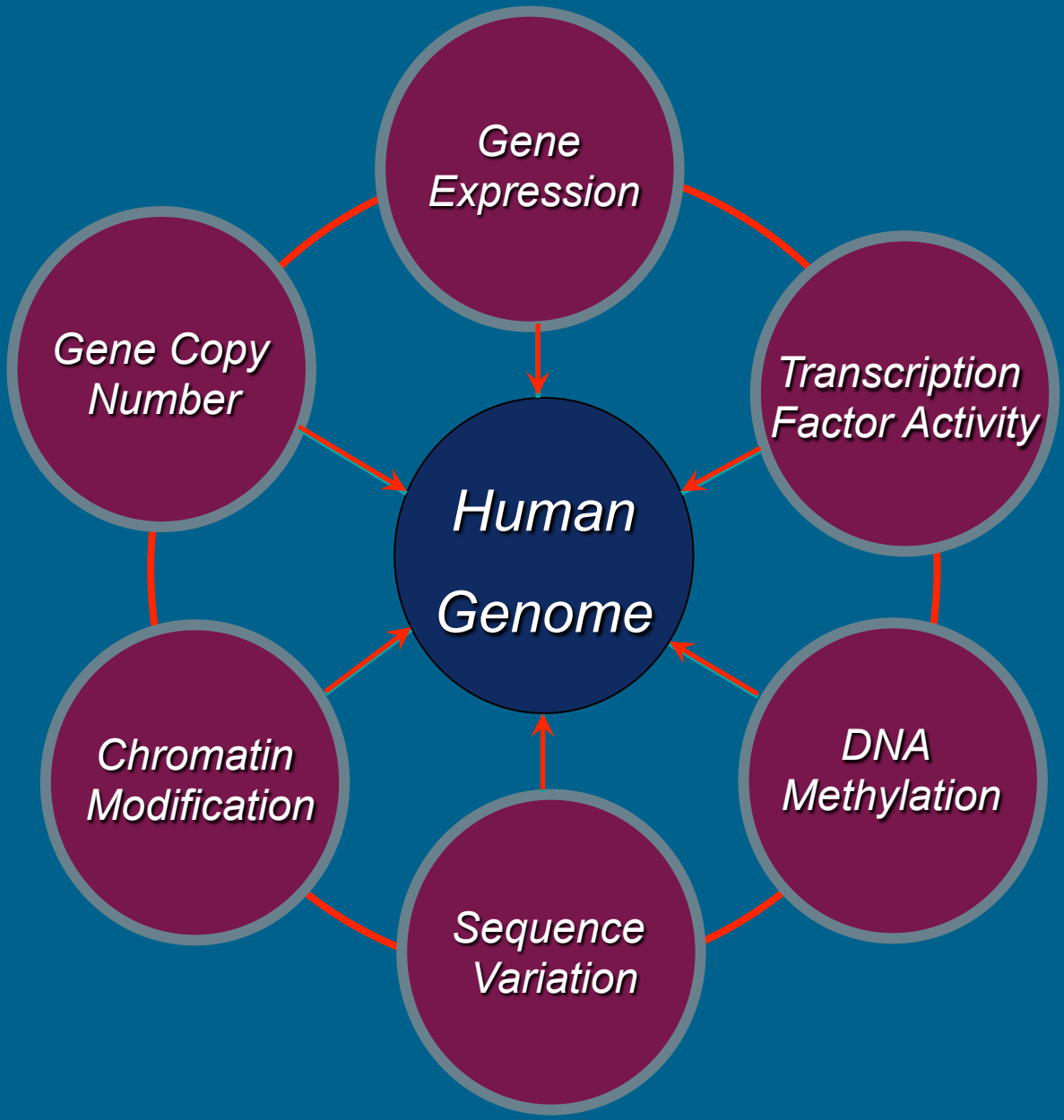
FROM DNA STRUCTURE TO THE HUMAN GENOME



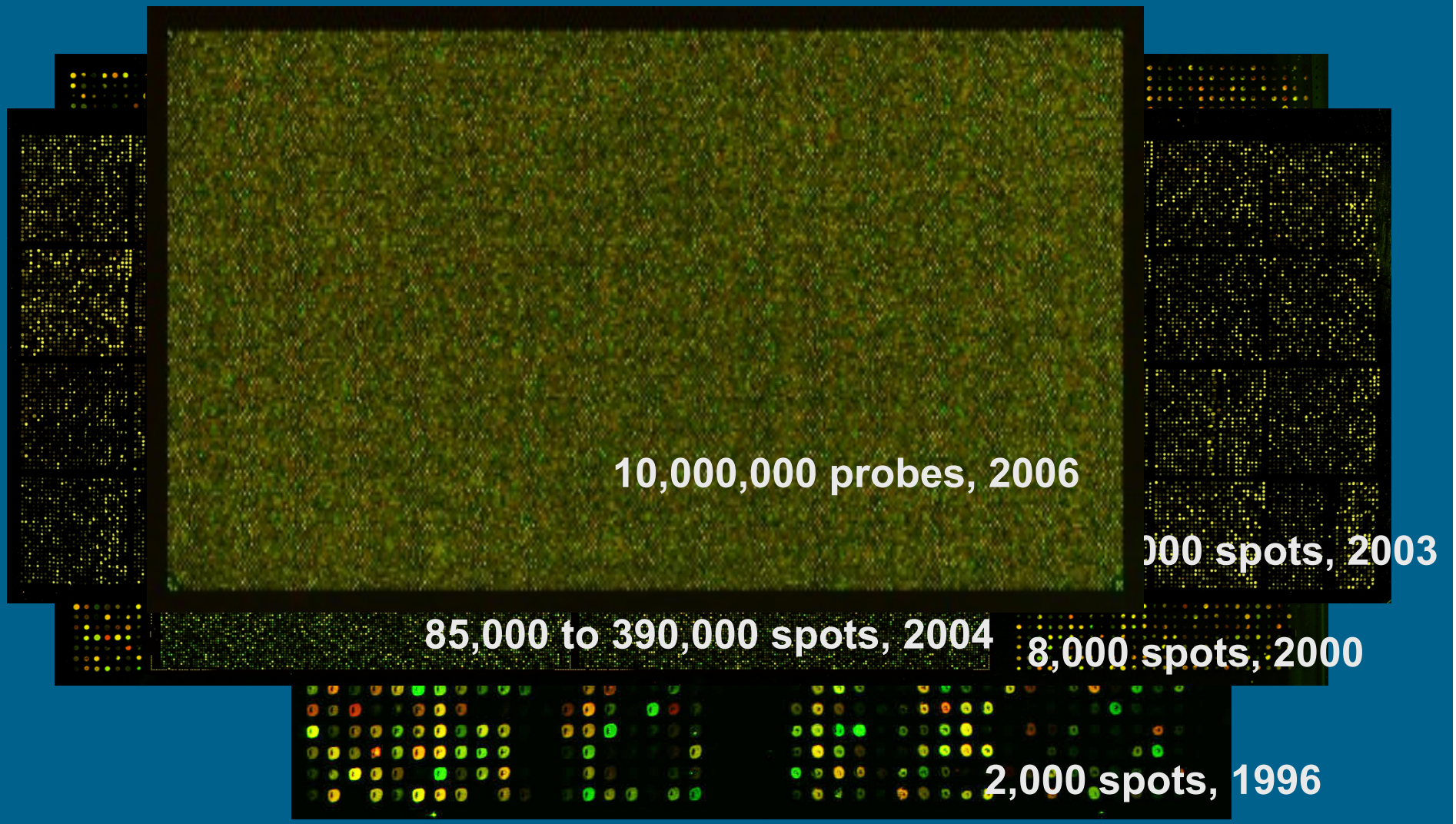
1953 UNDERSTANDING GENOME FUNCTION → 2001

TECHNOLOGY DEVELOPMENT

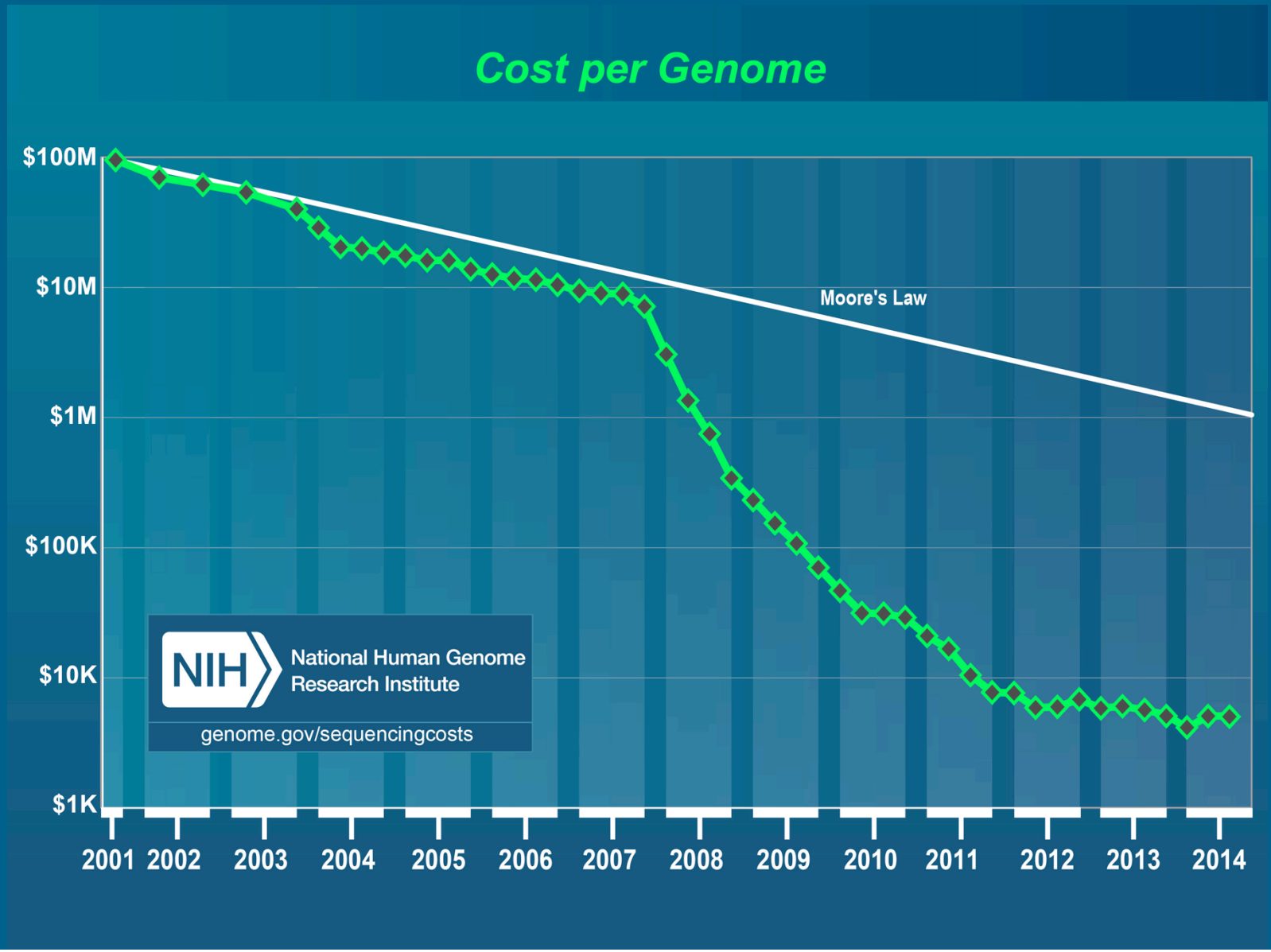
INTEGRATED GENOMICS



GENOME TECHNOLOGIES: MICROARRAYS



GENOME TECHNOLOGIES: DNA SEQUENCING



CANCER AS A GENOMIC DISEASE

"...in every cell there is a specific arrangement for inhibiting ...and definite chromosomes which inhibit division....
Tumors would arise if those inhibiting chromosomes were eliminated"

T. Boveri 1902

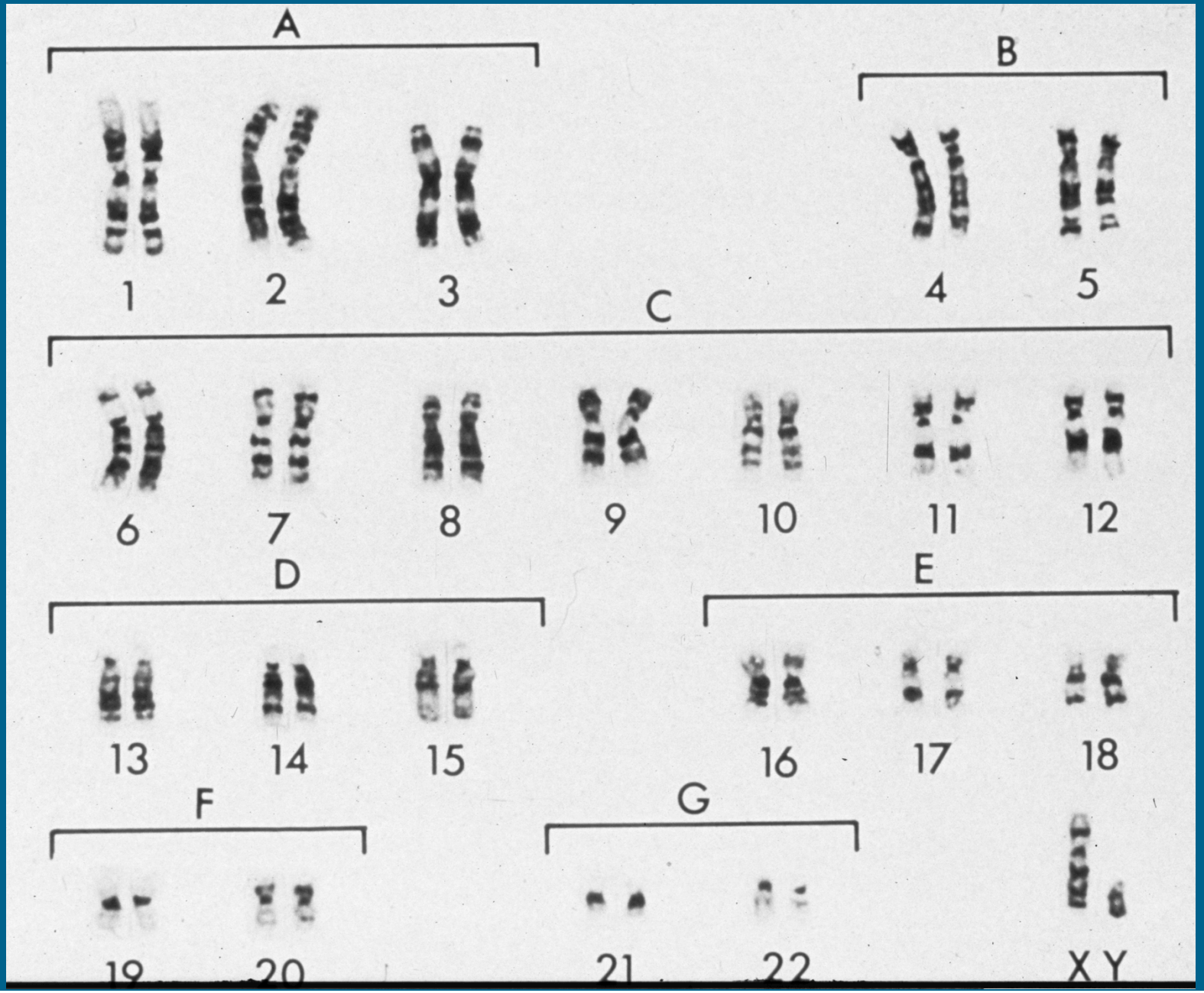
"What can be the nature of the generality of neoplastic changes... for the steplike alterations that they frequently undergo. A favorite explanation has been that oncogenes cause alterations in the genes of the body, somatic mutations as these are termed. But numerous facts, when taken together, decisively exclude this supposition. "

P. Rous Nobel Prize Lecture 1966

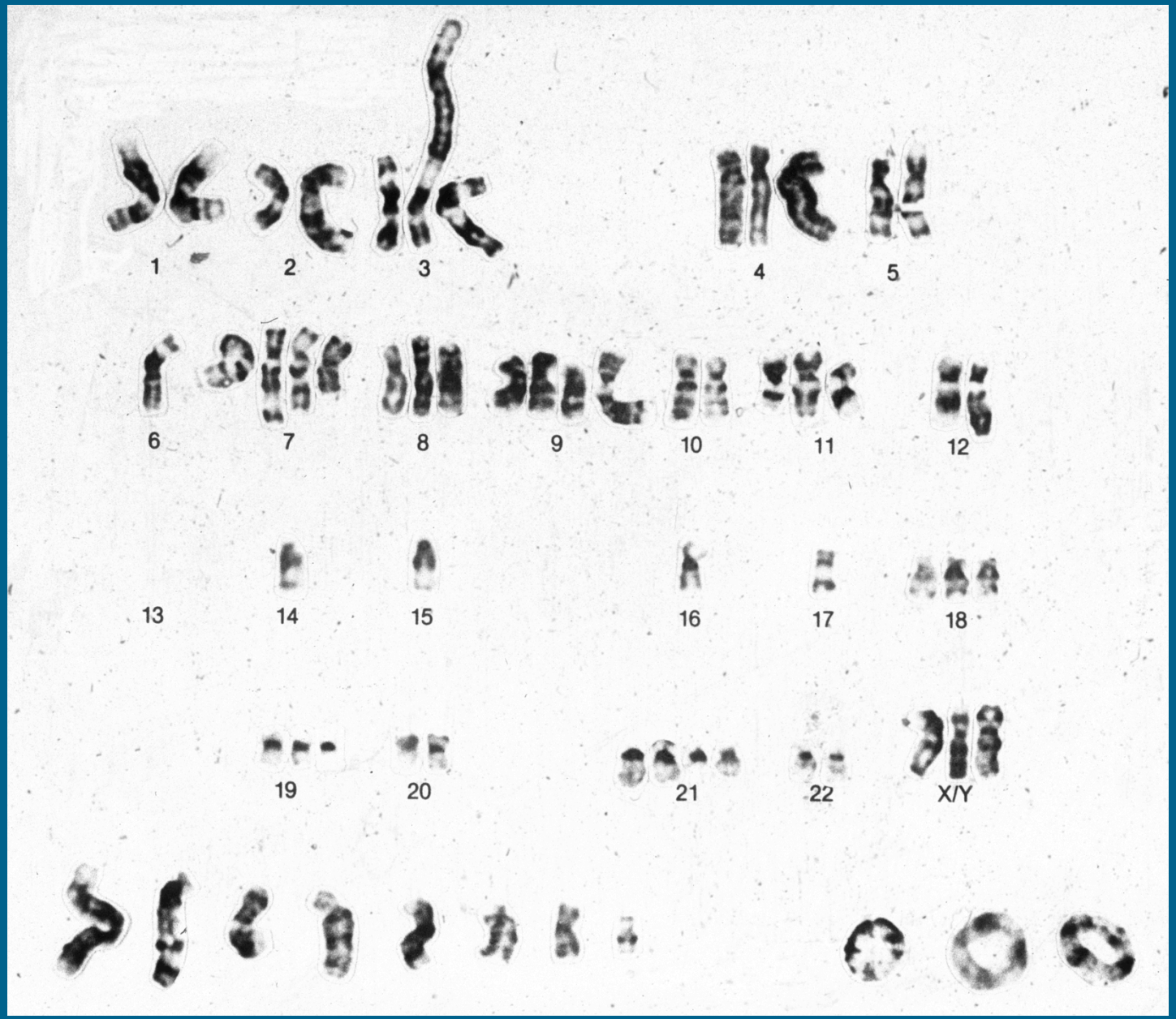
CANCER GENOME CHARACTERIZATION

It is taken as axiomatic that alterations in the cancer genome substantially determine the malignant phenotype, and that characterizing these will yield correspondingly substantial insights into tumor biology.

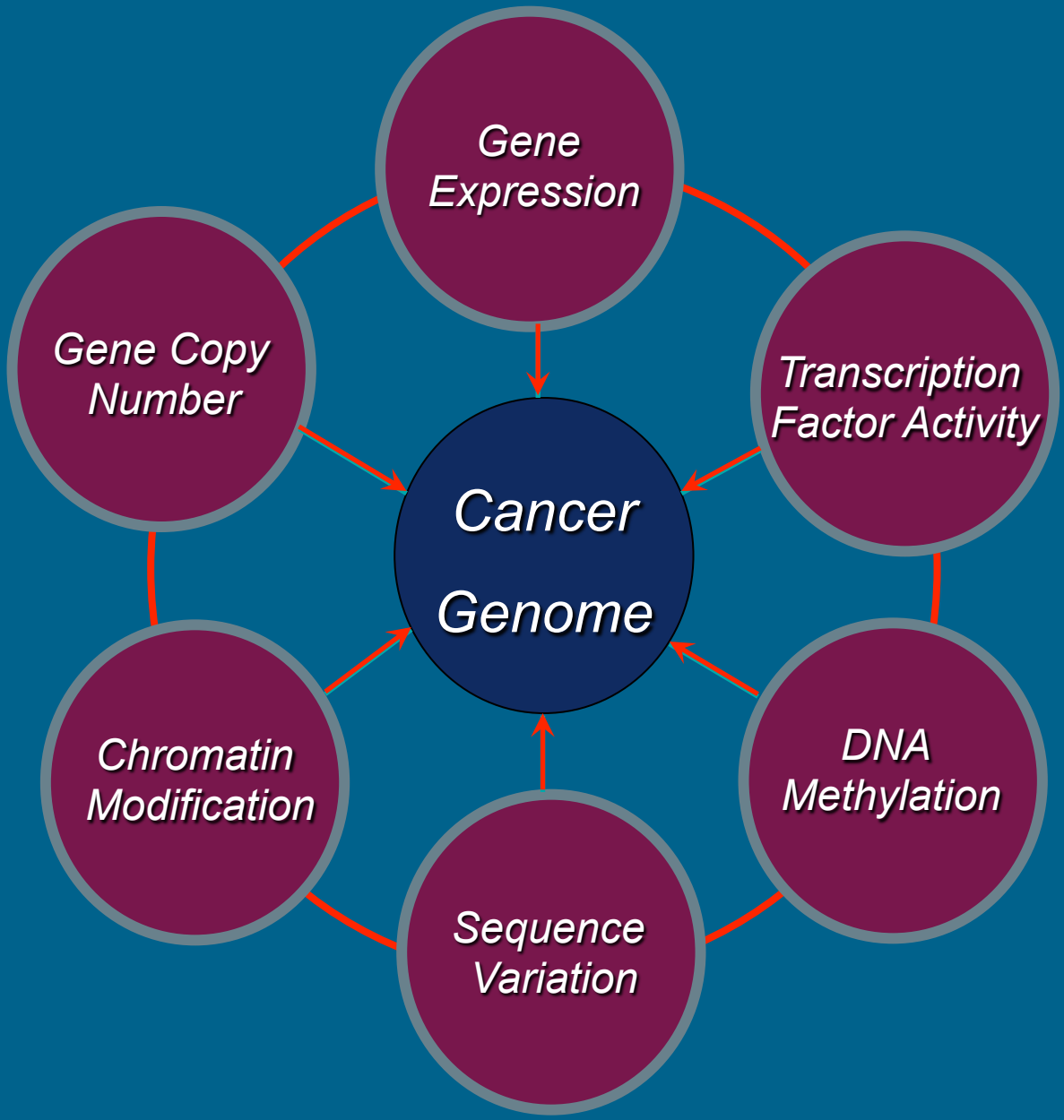
NORMAL CHROMOSOMES



CANCER CHROMOSOMES

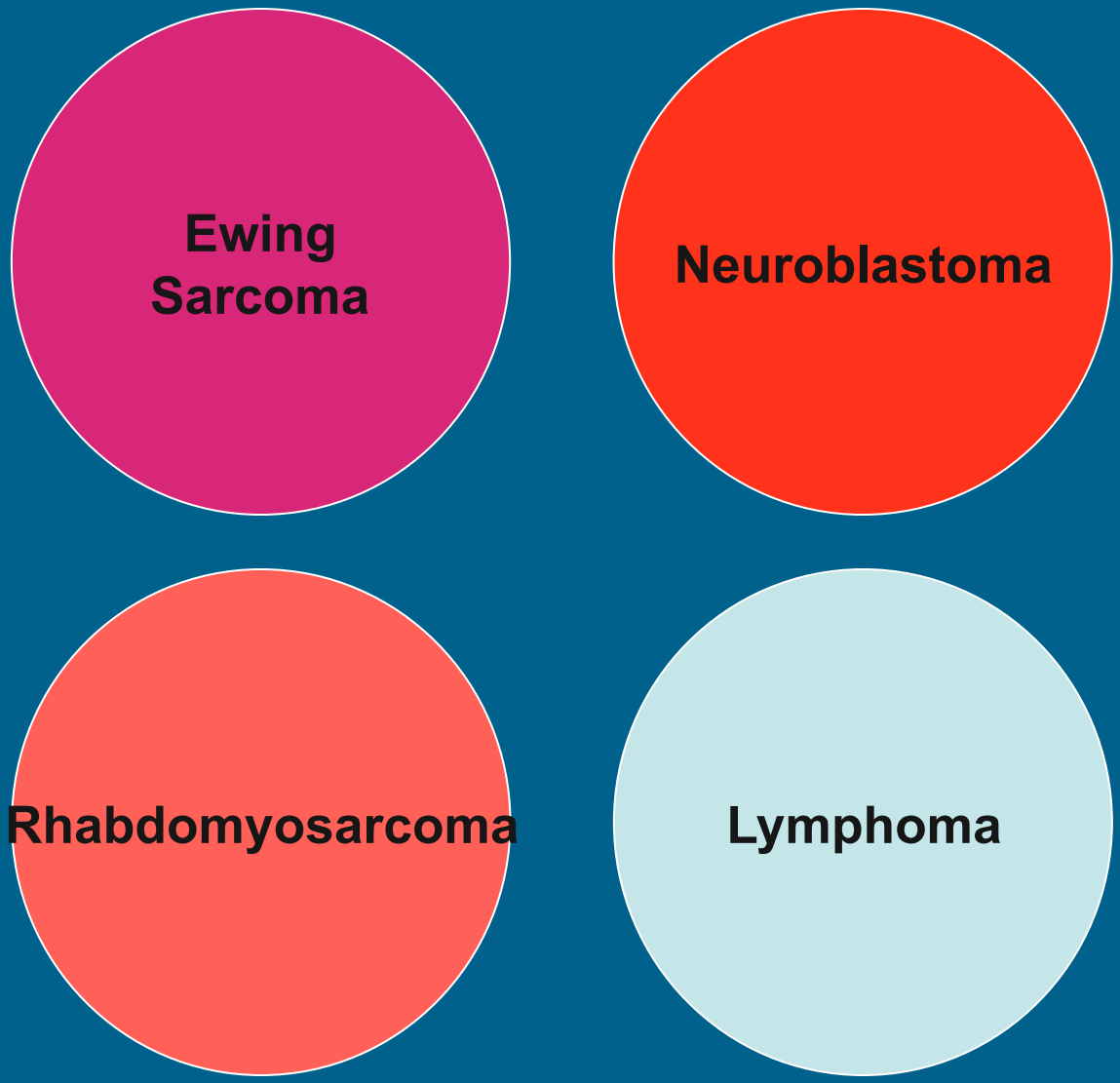


INTEGRATED CANCER GENOMICS



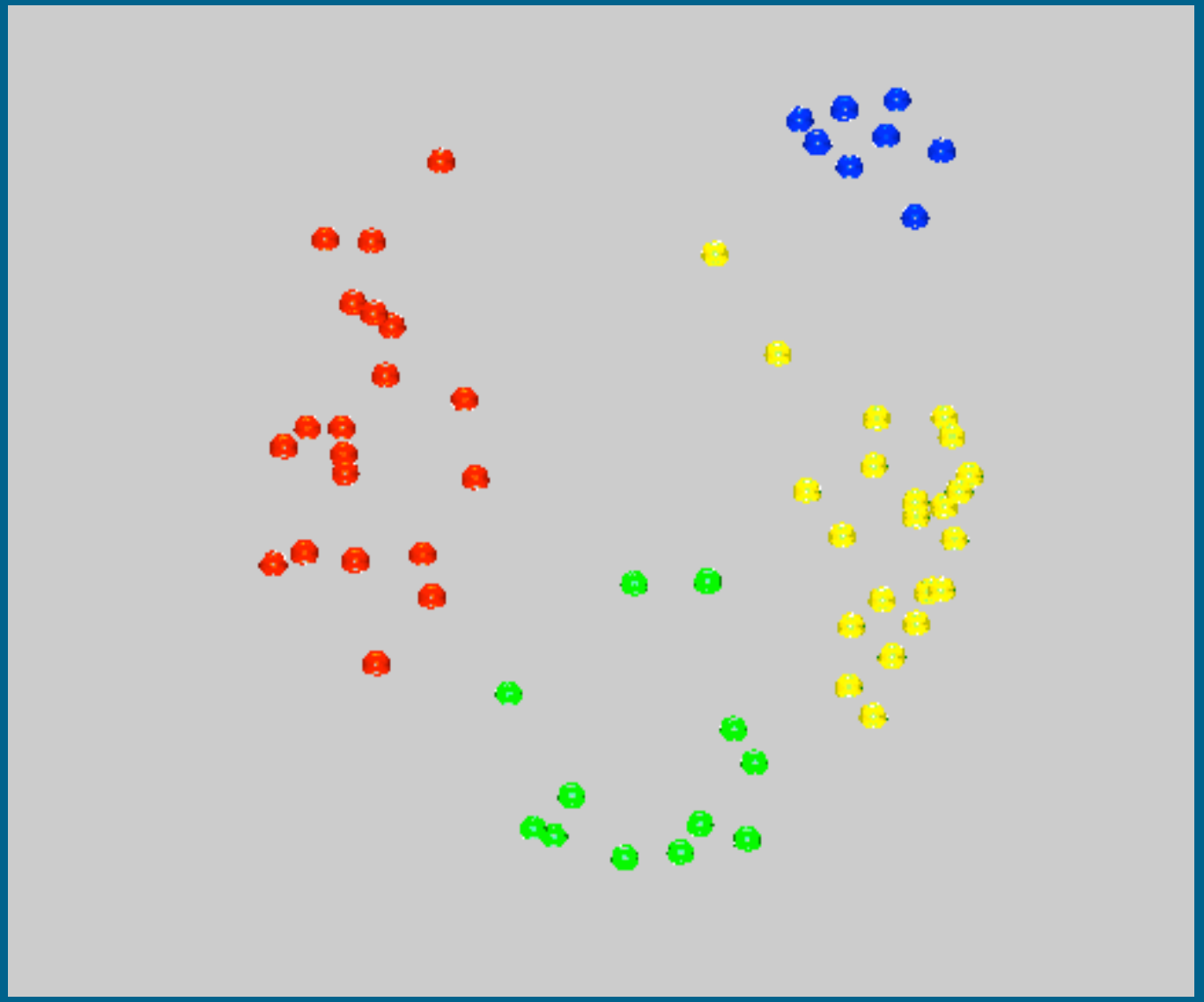
CAN CANCER TYPES BE DEFINED BY THEIR GENE EXPRESION PROFILE?

Tumor Classification Model: Small Blue Round Cell Tumors

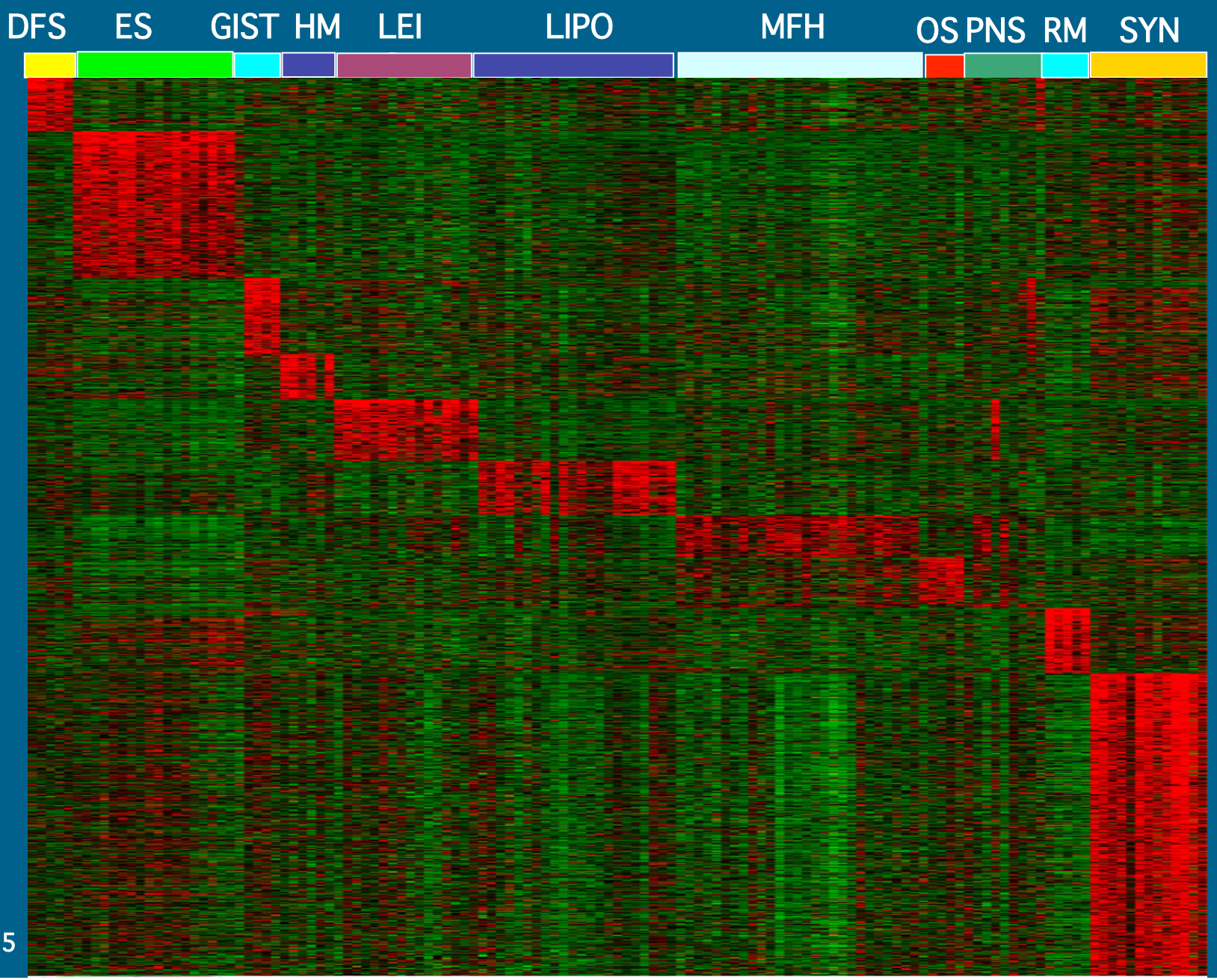


Khan J 2001

SEPARATION OF FOUR TUMOR TYPES BY GENE EXPRESSION

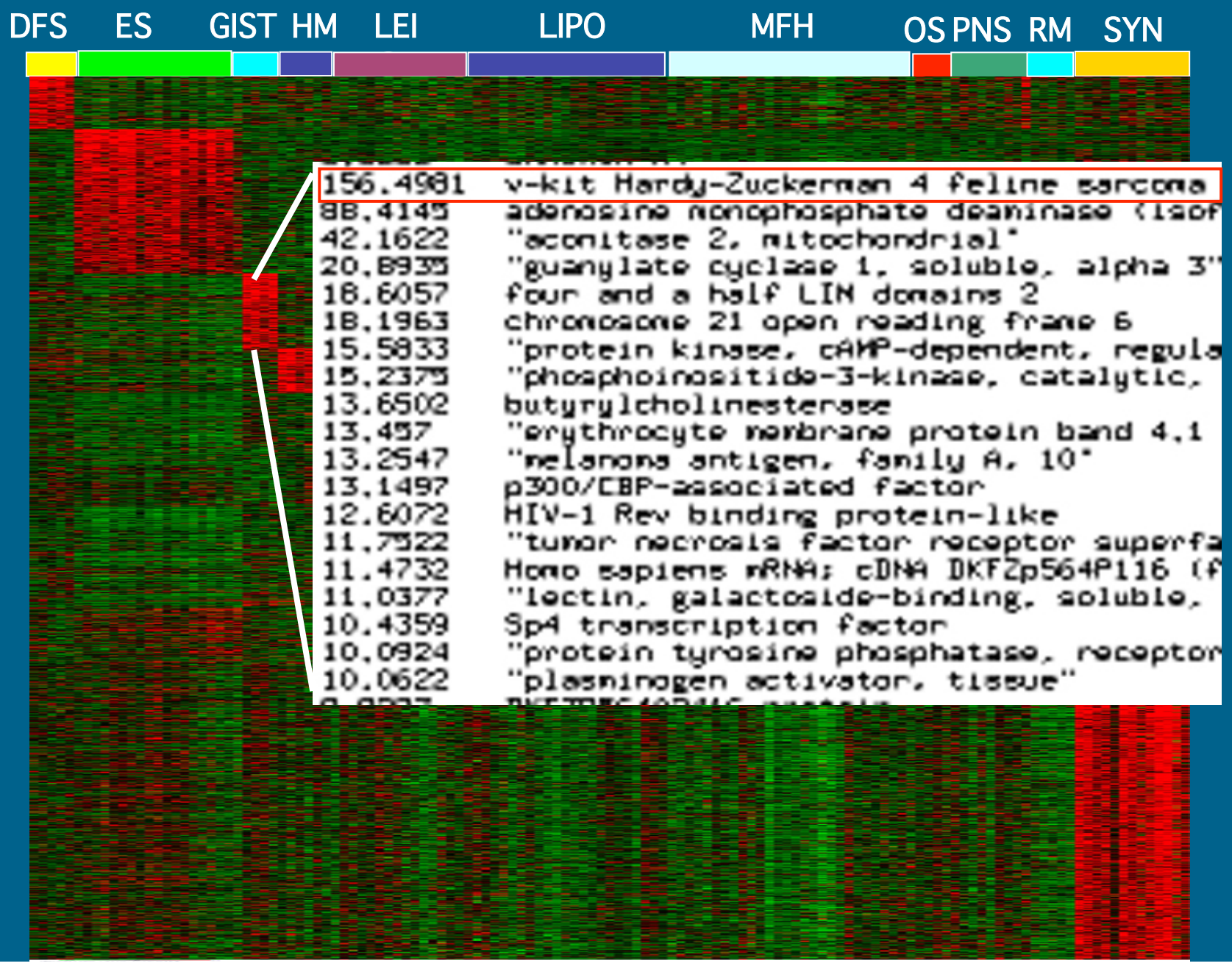


GENE EXPRESSION SIGNATURES OF 11 TUMOR TYPES



Baird K 2005

GENE EXPRESSION SIGNATURES OF 11 TUMOR TYPES



IMPLICATIONS OF TUMOR EXPRESSION PROFILING

- Powerful tool for class discovery.
- Data can be used to develop diagnostic and prognostic clinical tests.
- Identify genes for transition to clinical assays such as immunohistochemistry, flow cytometry, or Q-RT-PCR.

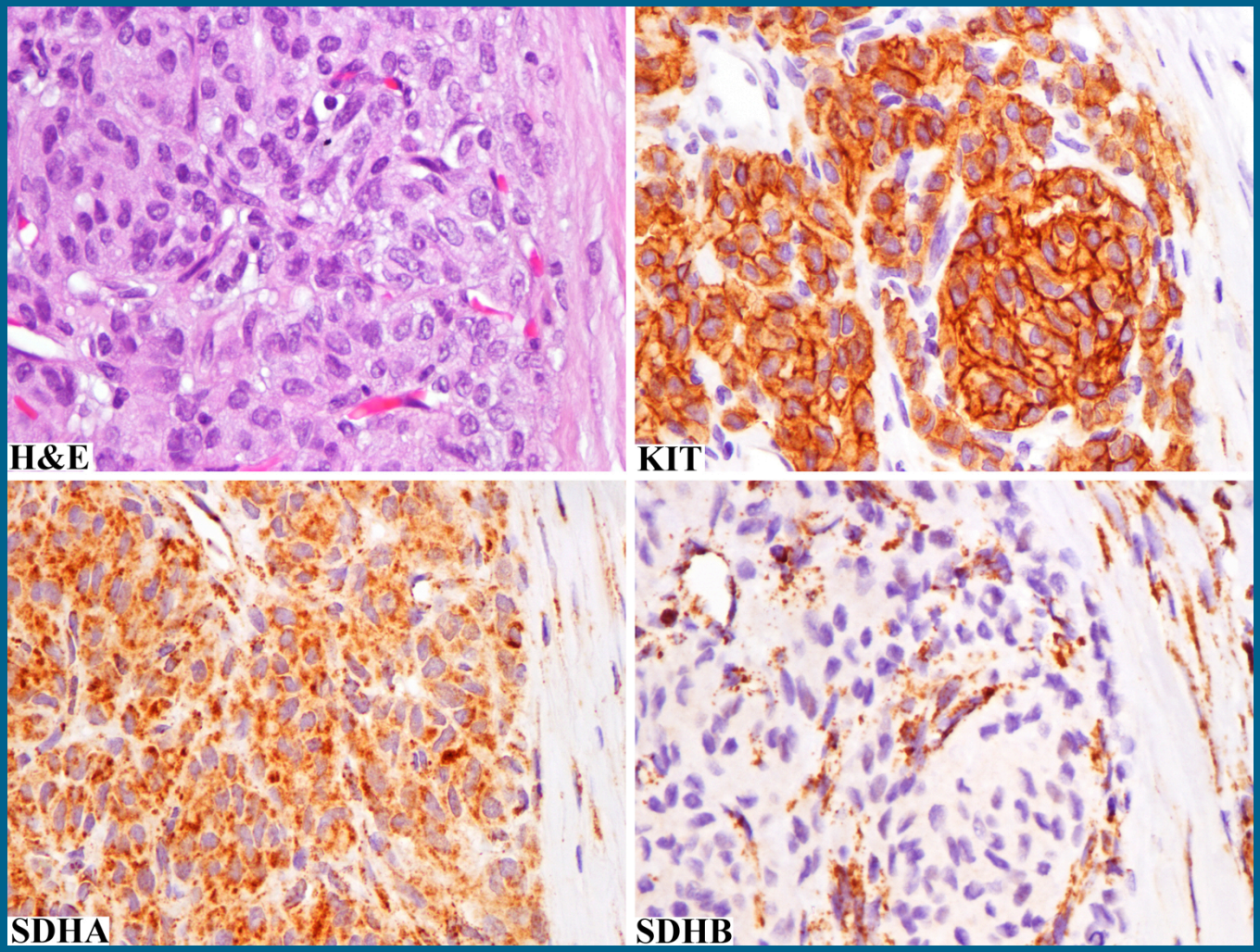
GASTROINTESTINAL STROMAL TUMOR (GIST)

- DERIVED FROM THE INTERSTITIAL CELLS OF CAJAL
- MODEL FOR TARGETED THERAPY (KIT, PDGFRA)
- NOT ALL GISTs CARRY THESE MUTATIONS.

GENETICS AND EPIGENETICS OF GASTROINTESTINAL STROMAL TUMOR

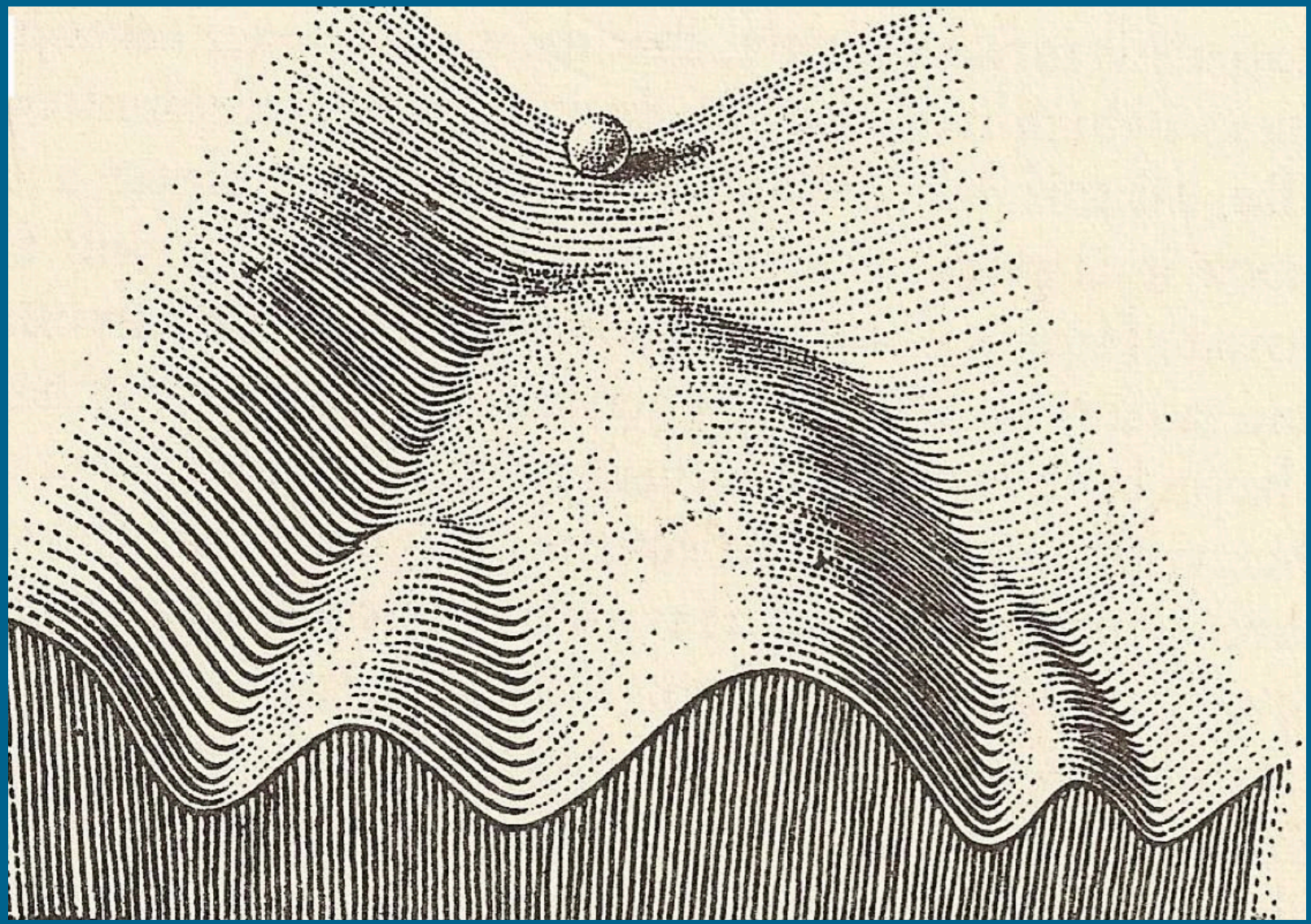
- SUBSET WHICH LACKS THESE MUTATIONS; OFTEN PEDIATRIC & HEREDITARY.
- MAINLY GASTRIC.
- HEREDITARY CASES MAY BE ASSOCIATED WITH OTHER TUMORS (PARAGANGLIOMA) AND MAY CAUSED BY MUTATIONS IN SDH subunit genes.
- SOME CASES FIT “CARNEY TRIAD” (GIST,PARAGANGLIOMA, PULMONARY CHONDROMA).
- NCI PEDIATRIC GIST CLINIC ESTABLISHED TO STUDY THESE PATIENTS.

SDHB DEFICIENT GIST



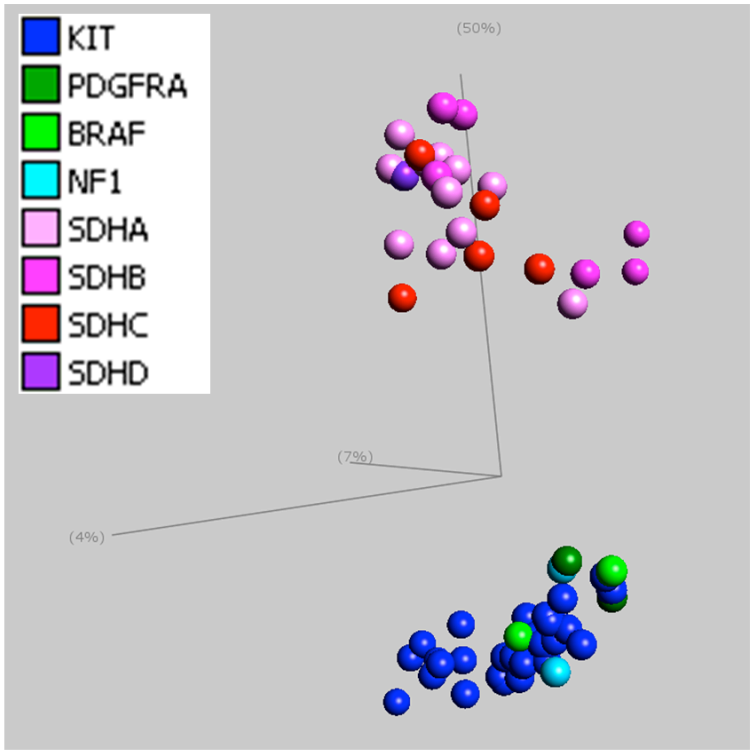
Miettinen et al. 2012

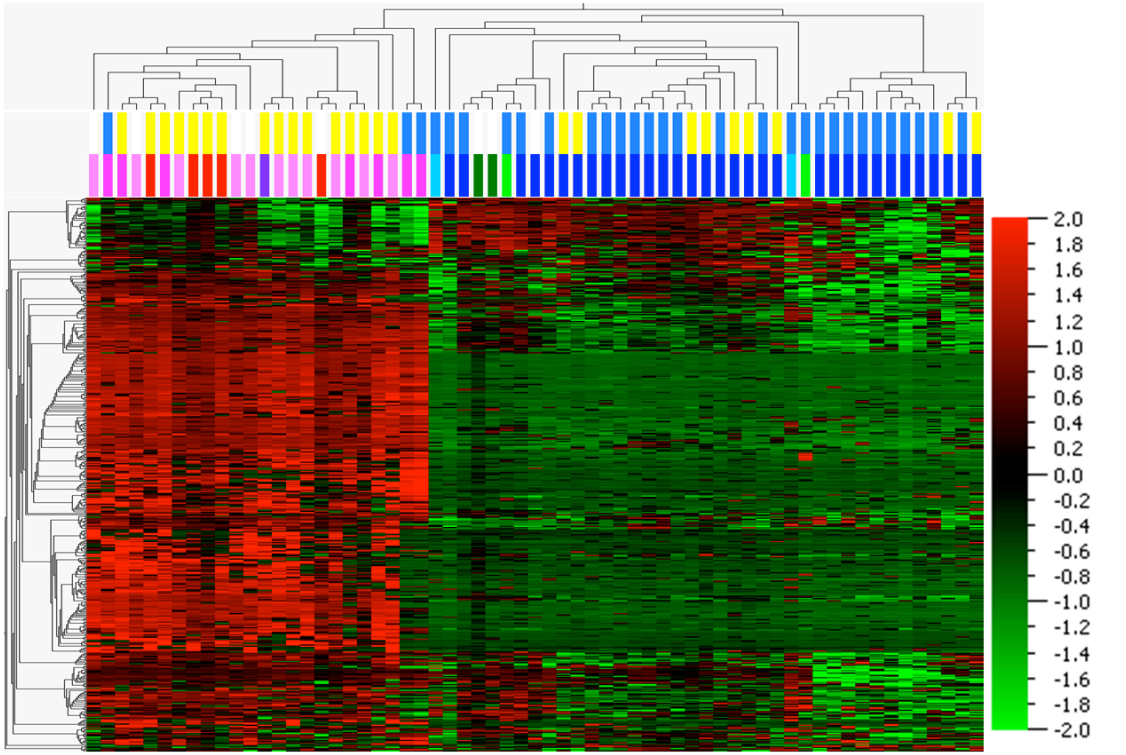
EPIGENETIC LANDSCAPE



WADDINGTON 1954

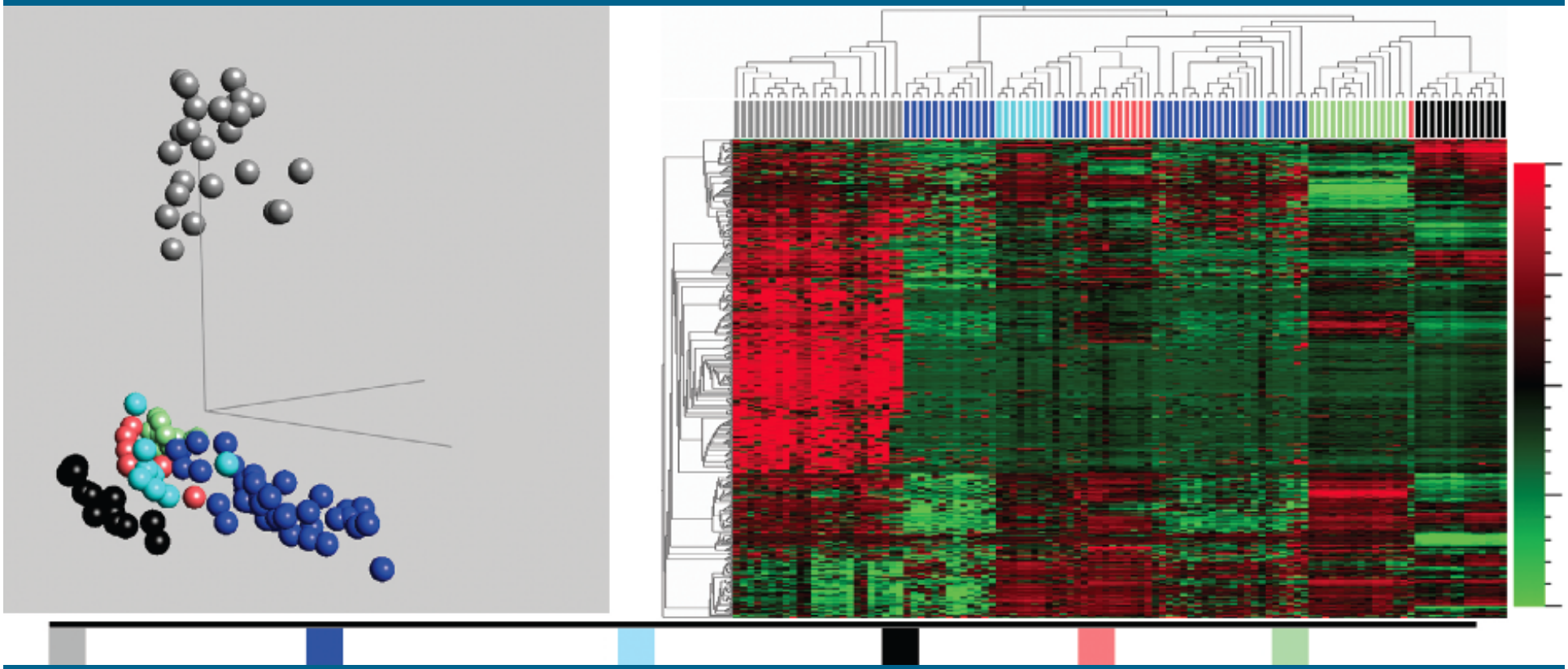
DISTINCT METHYLATION PATTERN IN SDH DEFICIENT TUMORS





Killian et al. *Cancer Discovery* 2013

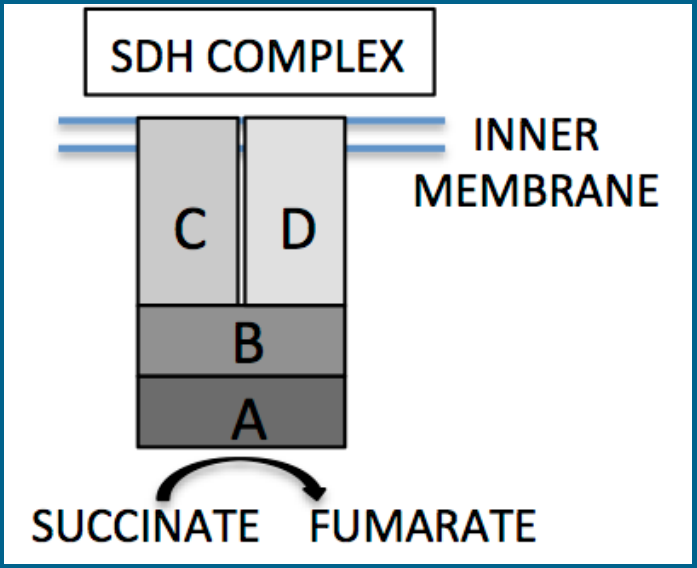
DISTINCT METHYLATION PATTERN IN SDH DEFICIENT TUMORS

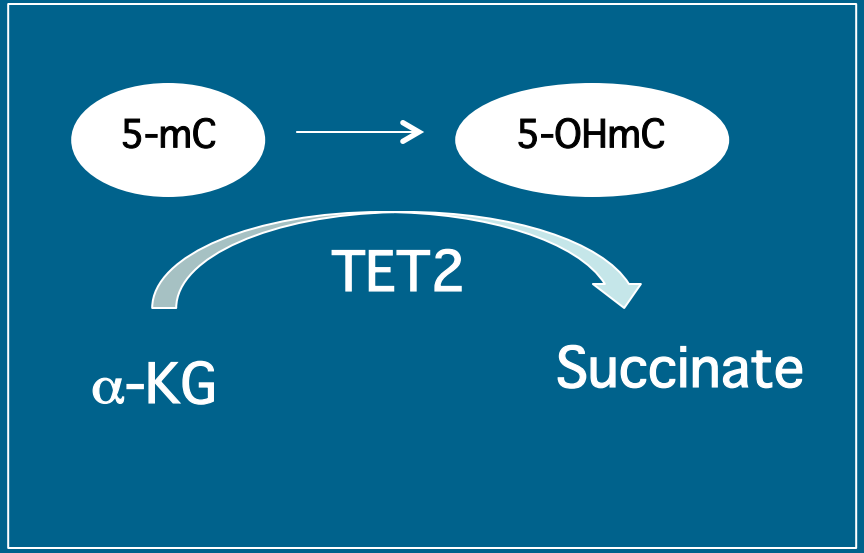


GIST SDHX GIST KIN. MUSCULARIS NEURON GASTRIC LYMPHOID

Killian et al. *Cancer Discovery* 2013

INTERACTION BETWEEN SDH DEFICIENCY AND TET2 DEMETHYLATION





Genetic Features of SDHx mutant tumors:

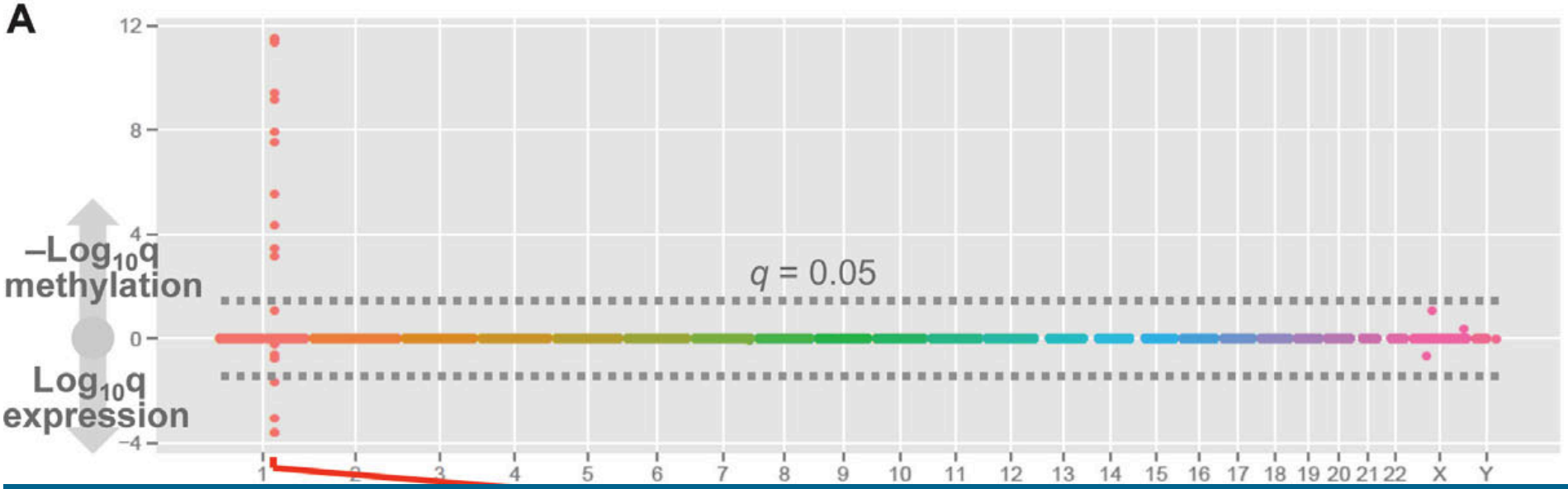
- **Deleterious mutation in an SDH subunit.**
- **Frequent LOH of second allele.**
- **Pervasive remodeling of the epigenome.**
- **Few copy number changes.**

Most SDH deficient tumors are readily classified by DNA sequencing, but there remains a significant group with no molecular diagnosis.

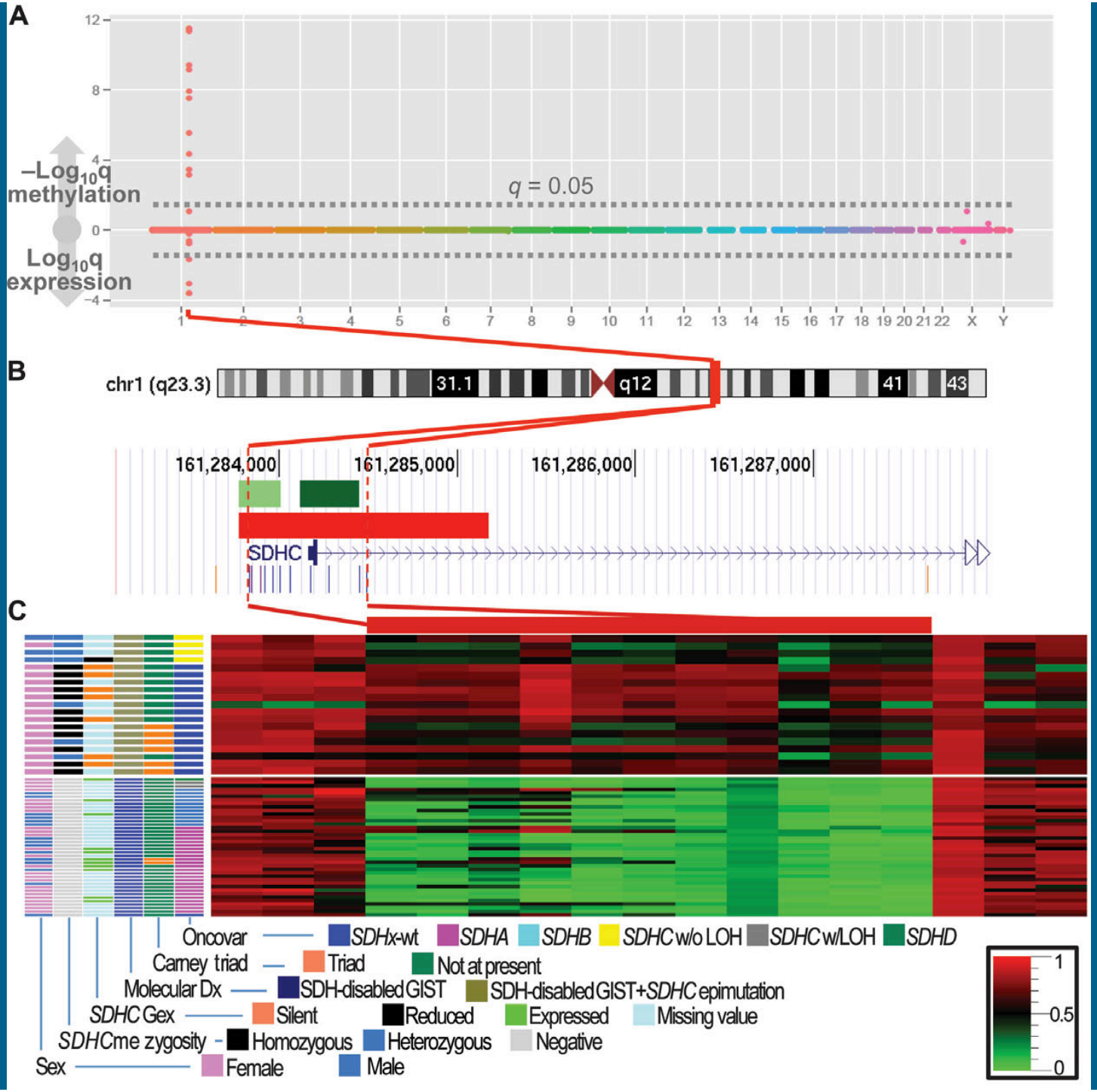
Question: What is going on in those patients?

Approach: Differential DNA methylation and gene expression analyses of SDHx wt vs. SDHx mutant tumors.

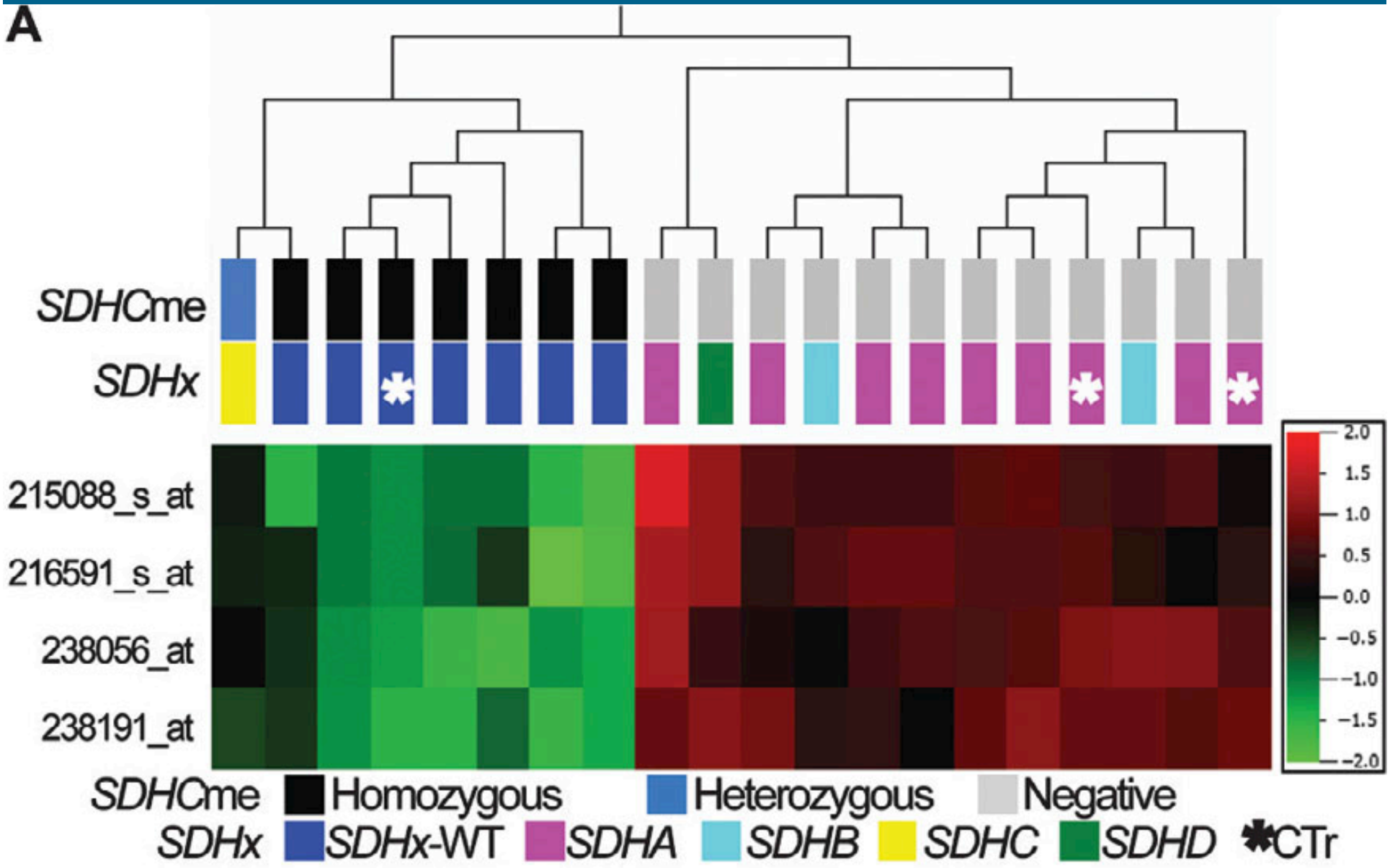
Whole Genome Janus Plot
Differential DNA Methylation and Gene Expression
SDHwt vs. SDHx



Killian et al. 2014


A
12
8
4
0
−4
−Log10q methylation
Log10q expression
q = 0.05
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y
B
chr1 (q23.3)
31.1
q12
41
43
161,284,000
161,285,000
161,286,000
161,287,000
SDHC
C
Oncovar
SDHx-wt
SDHA
SDHB
SDHC w/o LOH
SDHC w/LOH
SDHD
Carney triad
Triad
Not at present
Molecular Dx
SDH-disabled GIST
SDH-disabled GIST+SDHC epimutation
SDHC Gex
Silent
Reduced
Expressed
Missing value
SDHCme zygosity
Homozygous
Heterozygous
Negative
Sex
Female
Male
1
0.5
0

SDHC EXPRESSION SILENCING IN SDHx-WT GIST TUMORS



National Cancer Institute U.S. National Institutes of Health | www.cancer.gov

the NIH Pediatric & Wildtype GIST Clinic

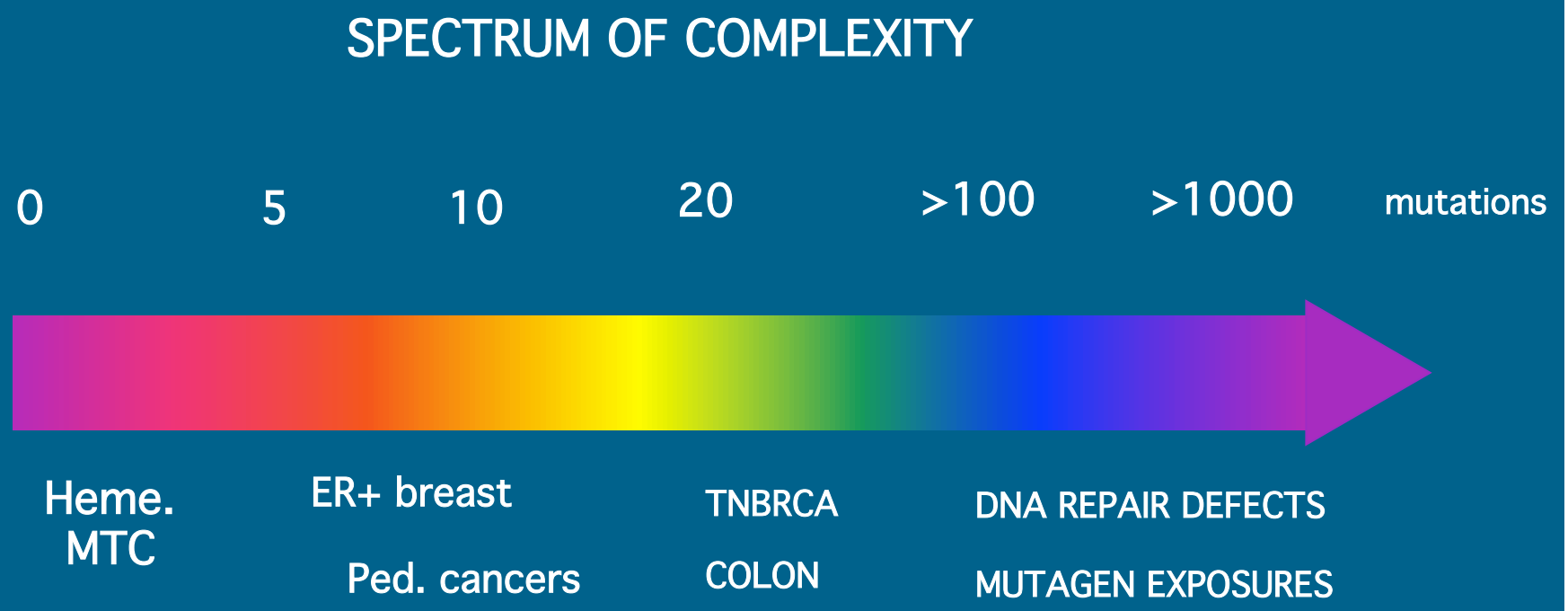
Home | The NIH GIST Clinic | Clinical Information | Research | Pediatric Collaboration | Links | Contact Us |

- INTEGRATION OF DNA SEQUENCE, DNA METHYLATION AND GENE EXPRESSION CONTRIBUTES TO A DEFINITIVE DIAGNOSIS IN THE MAJORITY OF SDH DEFICIENT GIST PATIENTS.

- IN TUMORS WHICH LACK SDHx MUTATION, SDHC IS TYPICALLY INACTIVATED BY A HIGHLY SPECIFIC EPIMUTATION.

- GIST PATIENTS WITH SDHC EPIMUTATION MAY BE SUSCEPTIBLE TO PARAGANGLIOMA AND PULMONARY CHONDROMA.

PROTEIN ALTERING MUTATIONS IN CANCER



SPECIFIC ALTERATIONS OF THE CANCER GENOME ARE TUMOR DRIVERS THAT POINT THE WAY TOWARD PRECISION THERAPY

- Chronic Myelogenous Leukemia

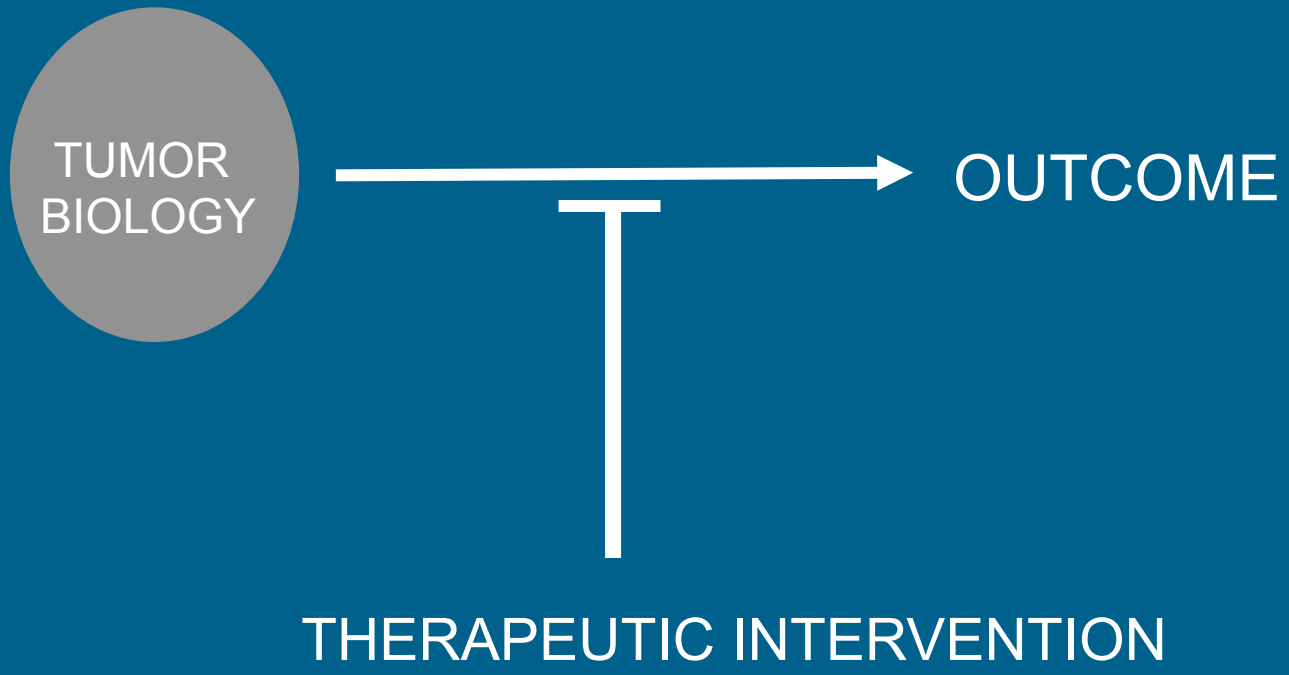
  Philadelphia chromosome (Nowell 1960)
  Translocation joining ch9 and ch21 (Rowley 1973)
  BCR-ABL fusion gene (Grosveld 1984)
  Imatinib treatment (Druker 1996)

- Does CML define a paradigm for cancer cure?

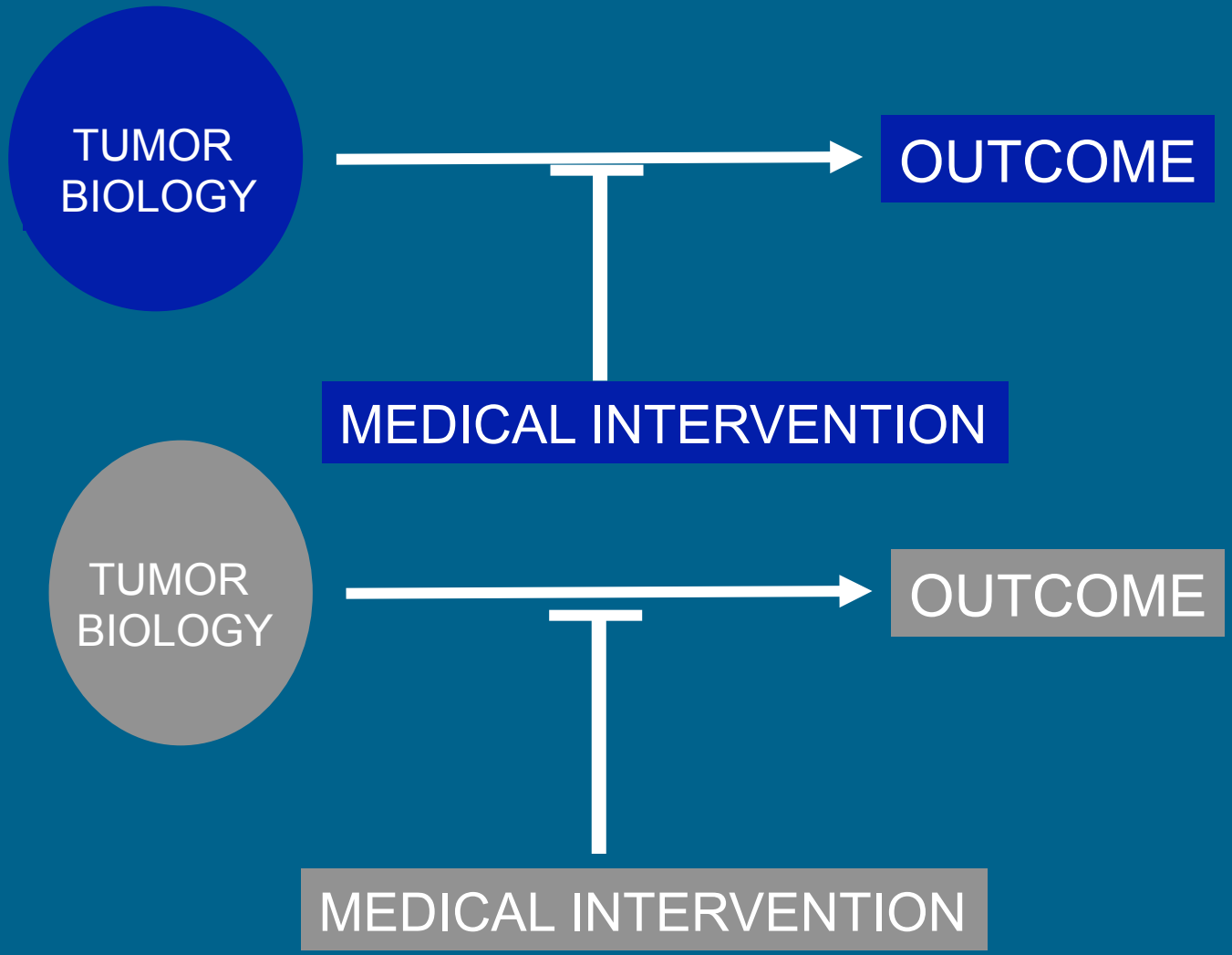
  -Specific driver gene needed for cancer cell survival.

  -Therapeutic agent which can target that gene.

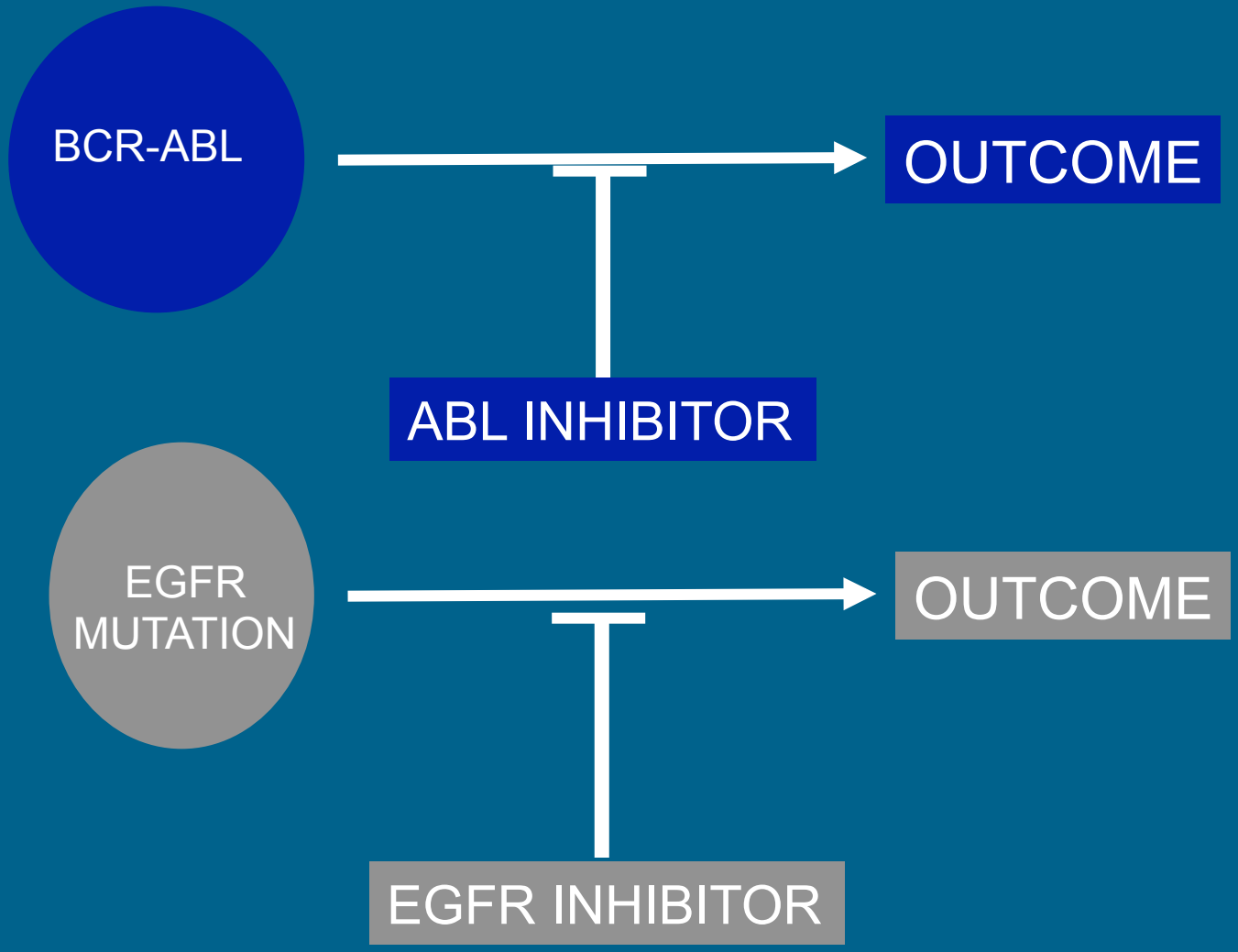
BIOLOGY AND OUTCOME



BIOLOGY AND OUTCOME



BIOLOGY AND OUTCOME



DOES THE CML PARADIGM GENERALIZE TO OTHER CANCERS?

Successes:

- Acute Promyelocytic Leukemia with PML-RARA fusion
- Gastrointestinal Stromal Tumor with KIT or PDGFRA mutations
- Breast cancers with ERBB2 amplification
- Colon cancers with EGFR mutations
- Lung cancers with ALK fusions
- Melanoma with BRAF mutations

But:

- In most common adult cancers, CML-like results are not achievable with a single drug even when a "druggable" target gene is present.

- A large proportion of cancers do not have a gene which fits the CML paradigm.

DOES THE CML PARADIGM GENERALIZE TO OTHER CANCERS?

Successes:

- Acute Promyelocytic Leukemia with PML-RARA fusion
- Gastrointestinal Stromal Tumor with KIT or PDGFRA mutations
- Breast cancers with ERBB2 amplification
- Colon cancers with EGFR mutations
- Lung cancers with ALK fusions
- Melanoma with BRAF mutations

And:

- Many of the altered genes that have been successfully targeted in cancers where they occur frequently are also observed at low frequency in diverse cancers and/or rare cancers.

- Could known targetable mutations be useful as molecular markers to guide precision therapy out of the disease context in which they were originally validated?

INCORPORATING DNA SEQUENCING INTO CANCER DIAGNOSIS

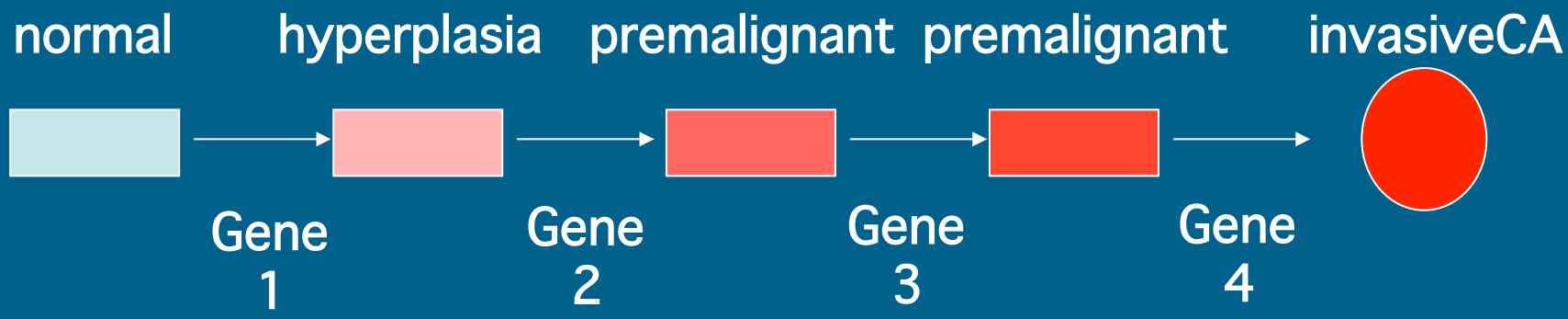
- It is now practical to sequence DNA from small clinical samples quickly and accurately.

- Sequencing can be on any scale from whole genome to individual genes.

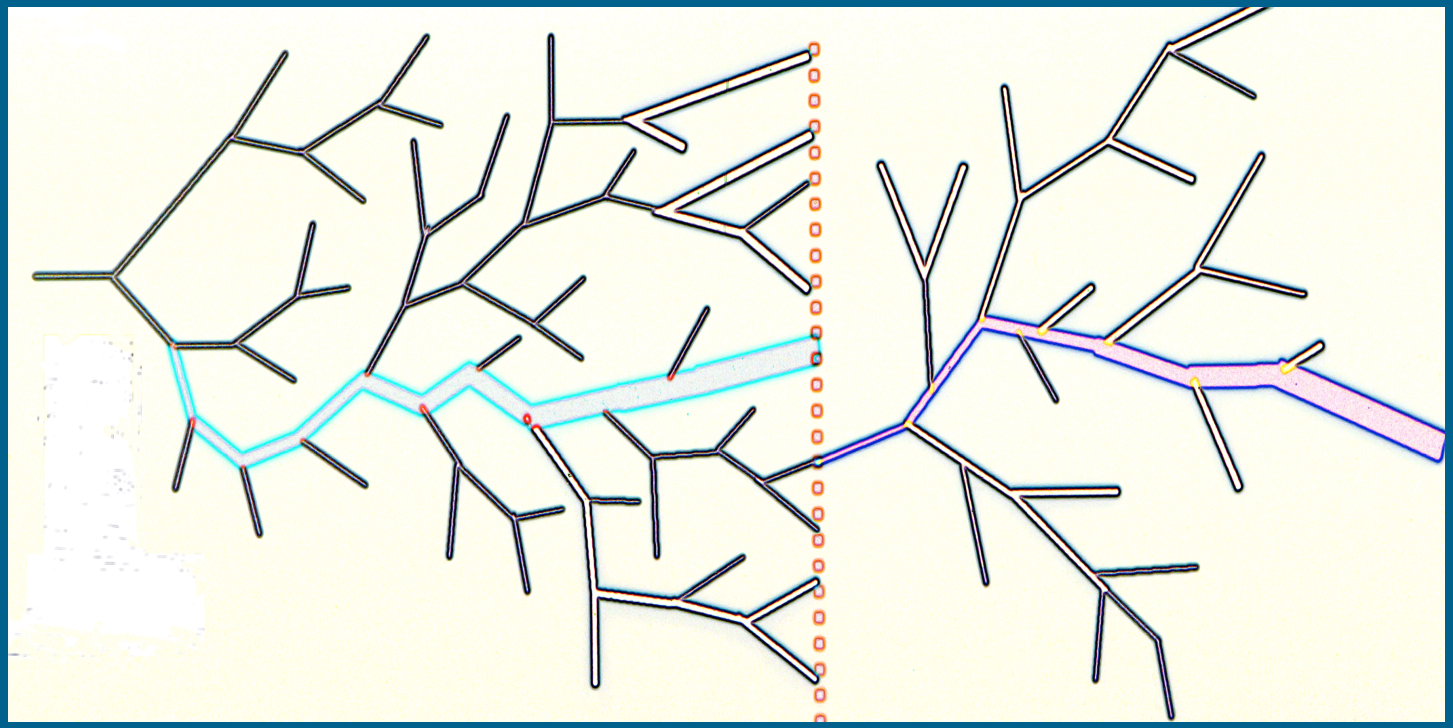
- Sequencing a panel of “driver” genes which could be targeted for therapy or which would aid definitive diagnosis is now clinically accessible.

- The broad utility of tumor DNA sequencing is currently a subject of ongoing clinical research, but sequencing is already standard-of-care in specific clinical situations.

LINEAR MODEL OF TUMOR PROGRESSION



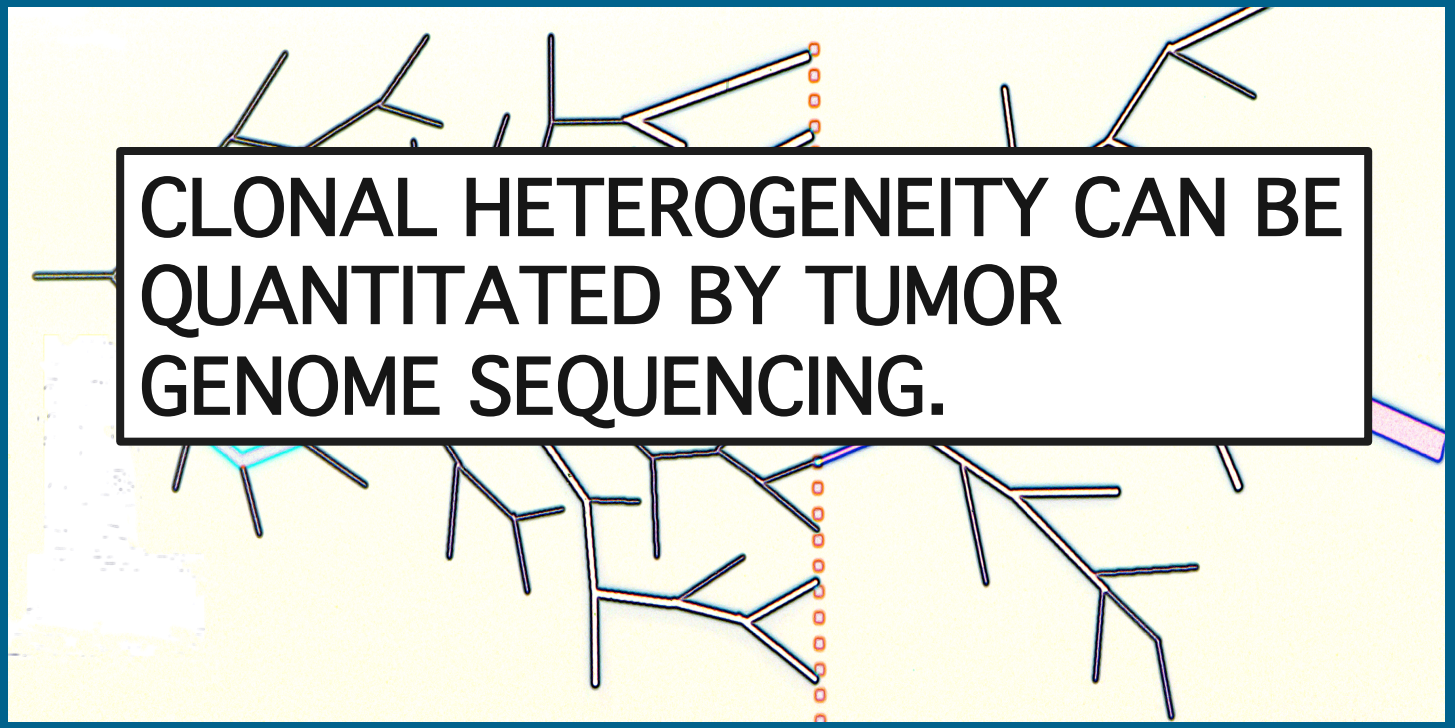
CANCER AS A BRANCHING CLONAL DISEASE



TUMOR GROWTH

RECURRENCE/METASTASIS

CANCER AS A CLONAL DISEASE



TUMOR GROWTH

RECURRENCE/METASTASIS

GENOMIC ONCOLOGY
EVALUATION AND LONGITUDINAL FOLLOW-UP

PRESENTATION:

Biopsy: T/N GENOME SEQ
RNA-Seq
Methyl-Seq

→

RECURRENCE:

Biopsy: T/N GENOME SEQ
RNA-Seq
Methyl-Seq

↓

- CLASSIFICATION
- OUTLIER GENE EXPRESSION
- COPY NUMBER/SMALL MUTATIONS
- NEOANTIGEN PREDICTION
- TRANSLOCATIONS
- PATHWAY/TARGET PREDICTION
- CLONALITY ANALYSIS
- GERM LINE PHARMACOGENOMICS

↓

- REPEAT ANALYSES AS AT DX
- RETARGET BASED ON ACTIVE CLONE(S)